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Research Paper

Matrix effect on leaching of Bisphenol A diglycidyl ether (BADGE) from epoxy resin based inner lacquer of aluminium tubes into semi-solid dosage forms

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ABSTRACT

To study the impact of different semi-solid dosage form components on the leaching of Bisphenol A (BPA) and Bisphenol A diglycidyl ether (BADGE) from the epoxy resin-based inner lacquer of aluminium tubes, the tubes were filled with different matrix preparations and stored at an elevated temperature. Despite compliance with the European Standards EN 15348 and EN 15766 on porosity and polymerisation of internal coatings of aluminium tubes, the commercially available tubes used in the study contained an increased amount of polymerisation residues, such as unbound BPA, BADGE and BADGE derivatives in the lacquer, as determined by acetonitrile extraction. Storage of Macrogol ointments in these tubes resulted in an almost quantitative migration of the unbound polymerisation residues from the coating into the ointment. In addition, due to alterations observed in the RP-HPLC chromatograms of the matrix spiked with BADGE and BADGE derivatives it is supposed that the leachates can react with formulation components.

The contamination of the medicinal product by BPA, BADGE and BADGE derivatives can be precluded by using aluminium tubes with an internal lacquer with a low degree of unbound polymerisation residues.

1. Introduction

Aluminium tubes are commonly used as container closure system for medicinal products of semi-solid dosage forms. The tubes are internally coated to avoid direct contact between the medicinal products and the metal. The protective coatings usually consist of Bisphenol A diglycidyl ether (BADGE)-based epoxy resins. These resins are known for their good mechanical properties and their chemical resistance [1].

BADGE resin is made from Bisphenol A (BPA) and epichlorohydrin. The polymerisation takes place under distinct curing conditions and in the presence of various additional reactants (hardeners, cross linkers, chain-stoppers, etc.) [2]. Simal-Gándara et al. [2] discussed the impact of the curing conditions on the degree of cross-linking and finally on a potential migration of unreacted compounds. For cans that are coated with BADGE-based epoxy resins and intended to be used to contain food, a specific migration limit for the sum of BADGE and derivatives (BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl, BADGE 2HCl and BADGE·HCl·H_2O) has been established by the European Commission [3]. The structures and reactions leading to these derivatives are outlined by Haverkamp et al. [4].

Previous studies have demonstrated that BADGE and its derivatives, as well as BPA, can also migrate into semi-solid topical formulations when they are stored in aluminium tubes coated with epoxy resins [4,5]. Extraction tests, by filling acetonitrile into empty aluminium tubes and storing them at 40 °C for 10 days, turned out to be a suitable method to estimate the amount of unreacted BPA and BADGE in the coating [4]. A plain variability was found in the extraction profiles among commercially available tubes from different vendors. Among them, one type of tubes (tube A, vendor A) resulted in the highest extractable amount of BPA, BADGE and BADGE derivatives. This was confirmed for two different lots of this tube type [4].

The migration of leachables from the coating into topical medicinal products of semi-solid dosage forms was examined by using appropriate migration studies [4,5]. In addition to the expected correlation between migration and storage temperature or duration, a distinct effect of the matrix on the extent of migration

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and on the leachable profile became evident. In particular, matrix polarity turned out to play a crucial role [4].

There is a wide variety of semi-solid medicinal products; some of them are single-phase systems, and others are multiple-phase systems. Often, emulsifying agents are used to stabilise emulsions of water and oil phases and to enhance dispersion/solubility of an active ingredient. Such medicinal products may also contain additional excipients, such as antioxidants, preservatives and/or excipients that can help to optimise the topical drug release rate, drug stability, and local tolerance [6]. The aim of the study was to evaluate the impact of certain matrix components on the migration of BADGE derivatives from the inside-coating of aluminium tubes into semi-solid medicinal products.

The European Committee for Standardisation (CEN) published two technical documents with test methods for evaluating the quality of internal lacquer of flexible aluminium tubes [7,8]. While EN 15384 describes a method to evaluate the porosity of the lacquer, EN 15766 provides a standard procedure for determining the polymerisation of the internal coating of the tubes. The validity of these tests for identifying aluminium tubes with a high amount of unreacted BADGE and BPA as polymerisation residues in the internal coating was also evaluated during this study.

2. Materials

2.1. Reference substances

Reference substances used for these studies were described by Haverkamp et al. [4]. The internal standard Bisphenol A di-3-hydroxypropylether (BADHPE), CAS No. [37353-75-6] was purified as described in [4].

2.2. Chemicals

Acetonitrile was HPLC gradient grade (Sigma–Aldrich, Steinheim, Germany). Unless otherwise mentioned, further reagents were of analytical grade. n-Heptane and methanol were obtained from Sigma–Aldrich (Steinheim, Germany). Ammonium formate, and formic acid were purchased from Fluka (Buchs, Switzerland), and acetic acid, acetone, sodium hydroxide and sodium sulphate anhydrous from Merck (Darmstadt, Germany). Sorbitan monostearate (Span™ 60) was bought from Sigma–Aldrich (St. Louis, MO, USA), and polyoxyethylene (20) sorbitan monostearate (polysorbate 60, Tween™ 60) from Caesar & Loretz, Germany. Water was obtained from a Milli-Q water purification system (Millipore, Molsheim, France). Solid phase extraction was carried out on Bakerbond C18 500 mg/3 mL columns (Mallinckrodt Baker, Deventer, The Netherlands). Polyethylene glycols (Macrogol) and all further matrix ingredients were of Ph. Eur. grade and purchased from Caelo (Hilden, Germany).

2.3. Aluminium tubes

Two kinds of tubes for pharmaceutical use (type A and type M) were purchased from two different vendors. For type A, 5 mL tubes of two batches (type A#1, type A#2) were used. Tubes of type M had a nominal volume of 10 mL. All tubes were internally lacquered with BADGE based epoxy resins (verified via AT-FTIR, data not shown).

2.4. Matrices

1.5 kg of each matrix was custom-made in a Unimix (Haagen & Rinau Mischtechnik, Bremen, Germany). *Einfache Augensalbe DAC* (*Eye Ointment*) and *Macrogolsalbe DAC* (*Macrogol Ointment*) were prepared according to [9].

For preparation of *Eye Ointment* white soft paraffin (60% w/w) and liquid paraffin (40% w/w) were stirred with 20 rpm while heating to approx. 70 °C until white soft paraffin was visually dissolved. The *Eye Ointment* was further modified by addition of either polysorbate 60 or sorbitan monostearate to a nominal concentration of 10% emulsifier related to the amount of original *Eye Ointment* (final concentration 9.1% w/w). For preparation of *Macrogol Ointment* the polyethylene glycols 300 and 1500 (both 50% w/w) were heated to approx. 40 °C and stirred with 20 rpm as well.

Once having reached visual homogeneity, heating was deactivated and all aforementioned matrices were stirred overnight.

Equally, 1 kg of a Macrogol/cetyl alcohol matrix was prepared containing Macrogol 400 (75.8%), Macrogol 1500 (4.1%), Macrogol 4000 (8.2%), cetyl alcohol (11.4%) and 0.5 M sodium acetate buffer pH 4.0 (0.5%).

2.5. Apparatus/HPLC methods

Leachables and extractables were analysed by use of RP-HPLC analysis (Dionex, Germering, Germany) using a binary gradient elution with 5 mM ammonium formate buffer and a methanol/acetonitrile (2:1) mixture coupled with fluorescence detection (λ_{EX} = 275 nm; λ_{EM} = 305 nm) as described by Haverkamp et al. [4] except for the Macrogol/cetyl alcohol matrix. Here, a slightly modified method was applied with an altered gradient containing methanol only starting isocratically with 60% methanol up to 15 min followed by a linear increase to 65% up to 22 min and to 70% up to 34 min. A Multospher 100 RP18-5 l, 250 × 4 mm column (CS-Chromatographie, Langerwehe, Germany) was used as stationary phase.

3. Methods

3.1. Extraction studies

The extraction studies were described by Haverkamp et al. [4].

3.2. Migration studies

The migration studies described in [4] were slightly modified to take into account high amounts of leachates from the aluminium tubes. Only one lot of tube A was used in one experiment to exclude effects of batch-to-batch variability.

Tubes of one supplier were manually filled, closed by folding and stored with closures down in a rack at 30 °C (intermediate) or 40 °C (accelerated), according to the current EU regulatory guideline for stability studies with drug products [10]. For reference, the matrix samples were simultaneously stored in closed glass containers at identical temperature conditions and protected from light. The Macrogol/cetyl alcohol matrix was additionally stored in glass containers after spiking with BPA, BADGE and its derivatives (at about 0.8 mg/kg matrix).

Sampling of tubes and blank matrices was done at defined intervals. Container tightness was checked by means of differential weighing. Samples of tubes were mechanically stressed once per week to simulate in-use conditions as described by [4].

3.3. Sample preparation for HPLC analysis

After removal of the semi-solid formulae out of the tubes, a quantitative extraction of the analytes from the matrices was necessary prior to HPLC analysis. For this purpose, the contents of two

tubes were pooled and further processed as outlined below. *Eye Ointment* was prepared as described by [4].

3.3.1. Eye Ointment + 10% Span™ 60

Aliquots of 5.0 g matrix, removed from the tubes, were weighed in flasks in triplicate and spiked with 1 mL acetonitrile containing 5.0 mg/L internal standard, giving a BADHPE concentration of 1.0 mg/kg. 20 mL n-heptane and 19 mL acetonitrile were added to the flask, and the matrix was suspended via vigorous shaking. In the case of incomplete suspension, the sample was sonicated (Sonorex RK 100 H, Bandelin, Berlin, Germany) for a maximum of 5 min to achieve a visually homogeneous suspension. Liquid–liquid-extraction was carried out using 2 × 20 mL acetonitrile, followed by freezing of the acetonitrile phase at about −20 °C for at least 2 h. Filtration with folded paper filters (Ø 90 mm, Schleicher & Schüll Nr. 595 1/2, Dassel, Germany) over sodium sulphate anhydrous, followed by rinsing with acetonitrile (2 × 5 mL) cooled down to −20 °C, removes sufficiently the emulsifier, which would hinder the subsequent evaporation by foaming.

The combined acetonitrile fractions were evaporated to dryness (40 °C) under vacuum and further treated as described for *Eye Ointment* in [4].

3.3.2. Eye Ointment + 10% polysorbate 60

Aliquots of 5.0 g matrix were weighed in flasks in triplicate and spiked with 2 mL acetonitrile containing 50.0 mg/L internal standard, giving a BADHPE concentration of 20.0 mg/kg. 20 mL n-heptane and 18 mL acetonitrile were added to the flask, and the matrix was suspended as described above. Further sample preparation was carried out as described under *Eye Ointment* in [4], except following the SPE treatment. Here, the combined eluates from the SPE were transferred into a 20 mL volumetric flask, 4.2 mL acetonitrile was added, and the flask was filled up with water (HPLC quality). The injection volume was 10 µL and 100 µL in order to fit the validated working range.

3.3.3. Macrogol Ointment

Aliquots of 2.5 g matrix were weighed in flasks in triplicate and spiked with 2 mL acetonitrile containing 50.0 mg/L BADHPE, giving a concentration of 40.0 mg/kg.

6.4 mL of acetonitrile was added to the flask and the matrix was dissolved by manual shaking. The solution was transferred into 20 mL volumetric flasks and brought up to volume with water. After filtration through 0.45 µm filters (Chromatfil PET 45/15 MS, Machery-Nagel, Düren, Germany), quantitation took place by injecting 10 µL and 100 µL in order to fit the validated working range.

3.3.4. Macrogol/cetyl alcohol matrix

Aliquots of 2.5 g matrix were weighed in flasks in triplicate and spiked with 40 µL of acetonitrile containing 50.0 mg/L BADHPE, giving a concentration of 0.8 mg/kg.

20 mL of acetonitrile was added to the flask and the matrix was suspended by vigorous shaking and by an ultrasound bath (see above in Section 3.3.1 for details). The suspension was stored at −20 °C overnight and then filtrated with folded paper filters (Ø 90 mm, Schleicher & Schüll Nr. 595 1/2, Dassel, Germany), followed by rinsing with frozen (−20 °C) acetonitrile (2 × 5 mL) in order to remove as much cetyl alcohol as possible. The combined acetonitrile fractions were evaporated to dryness (40 °C) under vacuum. The residue was transferred, with 6 mL of acetonitrile, in small portions, into a 20 mL volumetric flask and brought to volume with water.

3.4. Method validation

Analytical method validation was performed with the modified *Eye Ointments* and the *Macrogol Ointment* as described in [4]. The limit of detection (LOD) and the limit of quantification (LOQ) were determined separately for each matrix, based on recovery data in accordance with DIN 32645 [11]. For the qualitative investigations with the Macrogol/cetyl alcohol matrix, specificity/selectivity and LOD were tested in line with ICH Q 2 (R1) [12].

3.5. Test on porosity and polymerisation of internal lacquer

The tests were performed according to EN 15384 [8] and EN 15766 [7]. According to EN 15766, the coating was swabbed using cotton saturated with acetone. The cotton was inspected for colouration and the coating for fading or discolouration.

4. Results and discussion

4.1. Method validation

The quantification method for BADGE and its derivatives, including the preparation procedure of the semi-solid matrices, has already been successfully validated for selected ointments, creams and gels [4]. Linearity has been confirmed for the working range between the limit of quantitation (LOQ) and 500 µg/L of the analytes as BADGE, BADGE derivatives and BPA.

In order to verify the validation results and to exclude any interference of the method by the components of the matrices, the recovery of the analytes in *Eye Ointment* modified matrices and in *Macrogol Ointment* was also evaluated (Table 1). The results for precision, recovery as well as LOD and LOQ were slightly higher than determined in the matrices previously tested [4]. Nonetheless, the validation data, presented in Table 1, confirm the suitability of the preparation scheme to quantify BPA, BADGE and its derivatives in the selected matrices.

4.2. Physicochemical characterisation of the tubes

For the purpose of evaluating differences in the physicochemical characteristics of the inner lacquer of the two tubes, the porosity and polymerisation of tube A and tube M were tested in accordance with the EN Standards 15766 and 15384 [7,8]. The test results showed no differences, neither between the different lots of tube A (test on porosity and polymerisation) nor between tube A and tube M (test on polymerisation) (Table 2). All tubes fulfilled the requirements of EN 15766 and EN 15384.

However, relevant differences in the amount of polymerisation residues BADGE, BADGE derivatives, and BPA in the coatings became obvious when extracting the tubes by use of acetonitrile (Table 2). A significantly lower level of all extractables was found in tube M. Particularly, the level of extractable BADGE was determined to be more than 100 times lower in tube type M than in tube A. In addition, a clearly smaller amount of the BADGE derivatives studied was measured, as well as of BPA. These differences cannot be attributed to the smaller volume of tube A in relation to tube M. As indicated in Table 2, the ratio between wetted area and volume does not significantly differ between tube A and tube M. Instead, differences in the epoxy resin curing process are assumed to be responsible for this considerably different amount of extractables.

Difference in the amounts of extractables BADGE, BADGE derivatives and BPA was also detected among two batches of tube A (Table 2). Haverkamp et al. [4] discussed the curing process as the most likely cause for this difference. The certificates of analysis supplied by the tube vendor were related to the lacquer in general

Table 1
Exemplary validation data of the matrices spiked with 20, 100, 200 µg/kg (modified *Eye Ointments*, mean of $n = 3$ each) and 40, 200, 400 µg/kg (*Macrogol Ointment*, mean of $n = 3$ each).

Validation parameter	Compound						
	BADGE·2H$_2$O	BPA	BADGE·H$_2$O	BADGE·HCl·H$_2$O	BADGE	BADGE·HCl	BADGE·2HCl
Eye Ointment + Span™ 60							
LOD/LOQ	7.4/26	7.4/26.1	4.8/17.1	4.6/16.3	4.0/14.4	10.3/35.7 (∗)	18.1/61.7 (∗)
Recovery	117.2/105.3/106.2	116.7/111.4/115.6	97.0/101.5/101.3	107.8/98.6/98.0	94.9/91.2/88.7	85.5/86.3/84.7	60.2/83.7/89.6
Precision	4.9/2.2/1.9	9.8/5.8/1.9	9.0/5.1/1.5	7.0/1.5/2.5	5.4/2.4/2.2	10.4/4.7/2.5	14.0/9.0/2.5
Eye Ointment + Polysorbate 60							
LOD/LOQ	8.6/30.7	3.1/10.7	2.8/9.5	2.8/9.9	2.7/9.2	2.8/9.7	3.8/13.3
Recovery	102.8/104.3/102.8	100.0/102.1/103.0	96.8/100.1/100.4	95.6/100.7/100.5	94.9/101.4/103.0	93.7/102.8/102.9	98.2/101.5/102.0
Precision	9.9/2.7/1.8	0.6/0.5/0.3	0.9/0.6/0.3	1.3/<0.1/0.2	0.3/0.2/0.3	1.3/0.4/0.2	1.3/0.5/0.4
Macrogol Ointment DAC							
LOD/LOQ	10.3/36.6	6.7/24.2	14.9/52.4	9.3/33.1	5.0/18.1	5.6/20.0	7.4/26.6
Recovery	106.6/99.3/101.3	108.4/100.4/102.5	119.9/101.4/102.3	113.2/100.0/101.5	99.9/98.4/100.9	102.2/98.3/101.8	104.6/98.3/101.0
Precision	4.4/1.4/2.8	1.8/0.8/0.7	10.3/4.8/1.6	5.4/0.7/1.0	3.1/0.4/1.0	2.8/0.8/0.9	7.0/1.2/0.4

Recoveries at the different concentrations and precision (RSD) are given in percentage. Limits of detection (LOD) and limits of quantitation (LOQ), given in µg/kg, were calculated according to DIN 32645 [11]. Due to the absence of relevant peaks during migration study heteroscedasticity of labelled analytes (∗) was ignored.

Table 2
Physical properties and extraction study results of aluminium tube M in comparison with tube A.

		Tube A		Tube M
		Batch A#1	Batch A#2	
Dimension	Size	5 mL	5 mL	10 g (approx. 10 mL)[a]
	Wetted area during extraction (dm^2)[b]	0.1645	0.1645	0.2739
	Size/wetted area (mL/dm^2)	30.4	30.4	36.5
Physical tests	Test on polymerisation (EN 15766)	Complies	Complies	Complies
	Test on porosity (EN 15384)	Complies	Complies	Not tested
Extractables by acetonitrile extraction (mg/6 dm^2)[c]	BADGE·2H$_2$O	0.063 ± 0.007[d]	0.031 ± 0.006[d]	0.011 ± 0.002
	BPA	0.136 ± 0.017[d]	0.174 ± 0.019[d]	0.067 ± 0.004
	BADGE·H$_2$O	1.142 ± 0.111[d]	0.771 ± 0.042[d]	0.028 ± 0.009
	BADGE·H$_2$O·HCl	0.010 ± 0.002[d]	0.010 ± 0.001[d]	n.d.
	BADGE	10.468 ± 0.857[d]	5.635 ± 0.365[d]	0.032 ± 0.014
	BADGE·HCl	0.288 ± 0.024[d]	0.233 ± 0.015[d]	n.d.
	BADGE·2HCl	n.d.[d]	n.d.[d]	n.d.
Σ extractables BADGE, BADGE·H$_2$O, and BADGE·2H$_2$O, calculated as BADGE (mg/6 dm^2)		11.609 ± 0.864	6.396 ± 0.367	0.069 ± 0.016

n.d.: not detectable.
[a] Calculated based on the area wetted during extraction.
[b] Calculated for a cylindric column, wetted at bottom and coat.
[c] Mean ± standard deviations ($n = 5$), units in accordance with foodstuff legislation [17].
[d] Calculated based on data published [4].

and identical for both batches. However, the information from vendor A was based on analyses performed by an external laboratory after curing the lacquer onto suitable inert substrates under laboratory conditions. Thus, the certificates only confirm general suitability of the lacquer for coating purposes but do not reflect the batch-specific properties resulting from the specific curing process of the epoxy resin lacquer on the tube.

The impact of curing conditions on the cross-linking degree and eventually on the amount of polymerisation residues has already been discussed by Simal-Gándara et al. [2]. They stressed that residual monomers remain in the epoxy resin inner coating if the curing parameter in particular for BADGE-based coatings is not correctly selected. Crucial curing conditions include curing time and (high) temperature [2]. If these settings are not suitable for the used equipment or not adequately controlled, the curing process may be incomplete. Batch-to-batch variability, variability within batches as well as higher amounts of residual monomers could be the consequence.

The study results further indicate that compliance with the published standards on evaluation of the internal lacquer of aluminium tubes does not give assurance that the level of polymerisation residues in the coating is low. Appropriate extraction studies of aluminium tubes as described by Haverkamp et al. [4] are indispensable for assessing the level of residual monomers in the epoxy resin lacquer and cannot be replaced by applying the European Standards EN 15766 and EN 15348 [7,8].

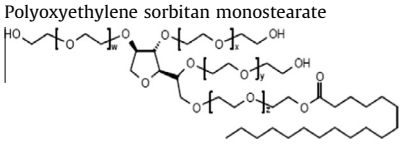
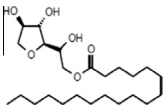
4.3. Formulation-dependent leaching of BADGE and its derivatives

In order to study whether the presence of an emulsifier in the medicinal product would have an impact on BADGE migration, typical formulations from German Drug Codex (DAC) were chosen as a model semi-solid dosage form. To see any difference concerning the influence on migration by hydro-/lipophilic balance we have selected two similar emulsifiers: the more lipophilic sorbitan monostearate (Span™ 60) and the more hydrophilic polyoxyethylene (20) sorbitan monostearate (polysorbate 60, Tween™ 60).

Both emulsifiers are well-described by pharmacopeia monographs and were alternatively added to the *Eye Ointment* formulation at a final concentration of approximately 10%. Chemical structures of the two emulsifiers are shown in Table 3. The spiked ointments were filled into aluminium tube A and stored up to 26 weeks at 40 °C.

Eye Ointment spiked with Span™ 60 did not show a significantly different leaching profile when compared with the results reported

Table 3
Emulsifiers Polysorbate 60 (Tween™ 60) and Span™ 60.

	Polysorbate 60 (Tween 60™)	Span 60™
Chemical name	Polyoxyethylene sorbitan monostearate	Sorbitan monostearate
Structure		
HLB	14.9	4.7

by Haverkamp et al. [4] for *Eye Ointment* without emulsifier (data not shown). Similar to the *Eye Ointment*, predominantly BADGE migrated into the modified matrix at a comparable low level (approximately 24 µg/kg) after 6 months of storage.

However, the presence of polysorbate 60 (Tween™ 60) in *Eye Ointment DAC* resulted in a considerable increase in the total amount of leachates in the ointment (Fig. 1). Already after two months of storage the amount of BADGE was more than 250 fold higher than in the unmodified ointment. Except for BADGE·2HCl, all BADGE derivatives, including BADGE·2H_2O, BADGE·H_2O, BADGE·HCl·H_2O, and BADGE·HCl as well as BPA, could be detected in the ointment spiked with polysorbate 60. BADGE was the predominant leachate, followed by BADGE·H_2O, which is formed after migration of BADGE by partial hydrolysis due to water present in the matrix [4]. According to the corresponding certificate of analysis, polysorbate 60 contained about 2.5% of water.

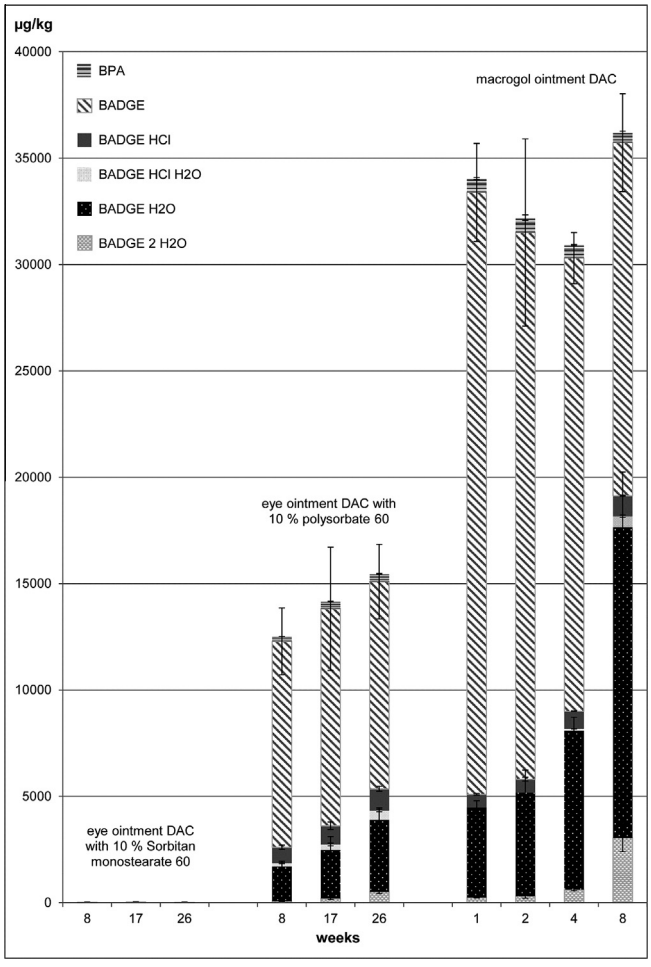


Fig. 1. Amount of BPA, BADGE and BADGE derivatives in *Eye Ointment* spiked with emulsifiers and in *Macrogol Ointment DAC* after storage in tube type A at 40 °C. Error bars reflect one standard deviation ($n = 3$).

A total sum of 15 mg/kg of all derivatives including BPA (calculated as BADGE), was quantified after 6 months of storage at the chosen storage conditions. As demonstrated in Fig. 1, leaching of BADGE derivatives from tube A into *Eye Ointment* spiked with polysorbate was nearly completed at the first sampling and testing point, i.e. after eight weeks of storage. The total amount of migrated substances did not significantly increase over the next four months of storage.

The results demonstrate that the presence of an emulsifier alone does not trigger the release of polymerisation residues from the coating. Thus, differences in the molecular structure of polysorbate 60 (Tween™ 60) and sorbitan monostearate (Span™ 60) were assumed to induce leaching. Beside the HLB-values (Table 3), the main difference between the two emulsifiers is the occurrence of oxyethylene units in polysorbate 60. In order to verify whether oxyethylene moieties in the matrix trigger leaching of BPA, BADGE and its derivatives, *Macrogol Ointment DAC*, a mixture of polyethylene glycol 300 and 1500 in a ratio of 1:1 (w/w), was filled into tubes of type A.

Fig. 1 shows that already after one week of storage at 40 °C, the amount of the polymerisation residues in the *Macrogol Ointment* was considerably higher than in the *Eye Ointment DAC* spiked with polysorbate 60, resulting in an overall amount of approximately 28 mg of BADGE per kg *Macrogol Ointment*. No relevant increase could be observed during the following weeks, thus demonstrating that the migration of BADGE and BADGE derivatives into the polyethylene glycol matrix had already reached equilibrium after storage at 40 °C for one week only. Hydrolysed BADGE derivatives and BPA were also present in the matrix (Fig. 1). In contrast to the overall amount of leachates, the amount of the hydrolysed derivatives further increased during storage, presumably due to hydrolysis of BADGE in the matrix, whereas the percentage of non-hydrolysed BADGE simultaneously decreased [4]. Furthermore, BADGE·HCl and BADGE·HCl·H_2O, but not BADGE·2HCl, were detectable in relevant concentrations.

The relation between the amount of polymerisation residues determined in tubes of type A by extraction studies with acetonitrile, and their amounts measured in the semi-solid products after storage, is demonstrated in Fig. 2. While addition of the emulsifier Span™ 60 did not increase leaching of polymerisation residues into the matrix, the presence of ethylene glycol moieties in polysorbate 60 considerably promoted the migration of BADGE and BADGE derivatives from the epoxy resin into the product. Up to 30% of the total extractable amount was determined after storage at 40 °C for two months (Fig. 2). A total amount of more than 90% was observed with *Macrogol Ointment* stored at 40 °C. But even when stored at 30 °C, a recommended storage temperature for long-term stability studies in compliance with ICH [10] up to 60% of the potential maximum amount of leachables was determined in the ointment when stored in tube A (Fig. 2).

Neither BADGE nor BADGE derivatives could be quantified in the ointment after storage at 40 °C for 8 weeks when *Macrogol Ointment* was stored in aluminium tube M (Table 4). All analytes (BADGE and BADGE derivatives) remained significantly below LOQ. Solely, BPA leached into the matrix. In-use conditions, by

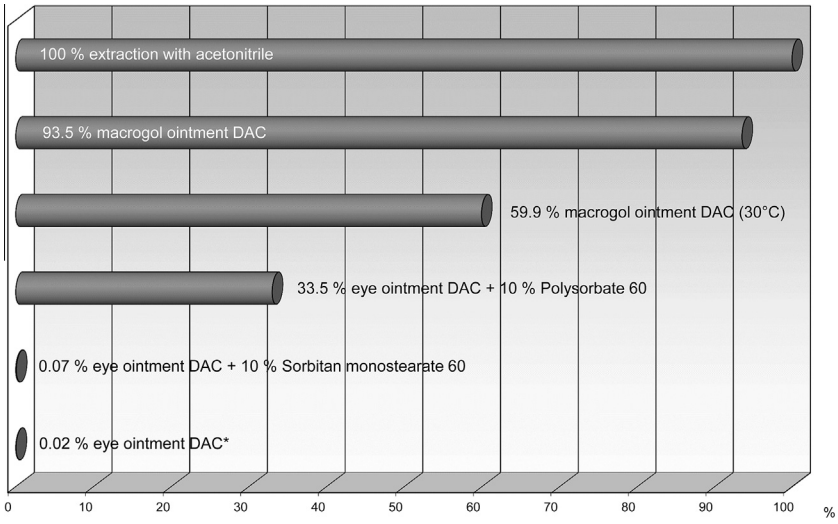


Fig. 2. Sum of leachates from tube A#2 after two months of storage at 40 °C, if not indicated otherwise, in relation to the sum of extractables determined by extraction with acetonitrile (7 days, 40 °C) set as 100%. *stored in tube A#1.

applying mechanical stress (periodic squeezing of the tubes), resulted in a slight increase of up to 130 µg BPA per kg *Macrogol Ointment*, which was still four times lower than with tube A (data not shown).

Oxyethylene-like structures within a semi-solid medicinal product appear to have extractive properties resulting in a nearly quantitative release of the polymerisation residues from the inner lacquer of aluminium tubes. This already leads to a considerably high level of leachates in the medicinal product after only a short time of contact at recommended storage temperature. Contamination of a semi-solid product containing components with oxyethylene structures can be precluded when aluminium tubes are used with a low level of extractable polymerisation residues, as in tube M.

4.4. Impact of leachables on product purity

Additionally, a stability study was initiated in order to evaluate the impact of the aluminium tubes on semi-solid formulation purity. For this purpose, a Macrogol/cetyl alcohol matrix used in commercially available drug products was prepared and filled in parallel into tubes of type A and M and in glass containers for reference. All containers were stored at 30 °C. Samples were pulled at several points of time and analysed using RP-HPLC.

In Fig. 3, the chromatograms of the Macrogol/cetyl alcohol matrix stored in tube A (line A) and in tube M (line B) are presented after 19 days of storage. The chromatogram of the matrix stored in tube A showed a high number of peaks, most of them with considerable peak areas whereas only a very few minor peaks were observed with the matrix stored in tube M. Compared to the chromatogram of a standard solution (containing all studied compounds; Fig. 3, line C), all peaks could be clearly detected in the chromatogram of the matrix after storage in tube A but not after storage in tube M. This finding confirms the results described above for *Macrogol ointment*.

Besides the peaks concurrent with the standard solution, numerous additional peaks appeared in the chromatogram of the matrix from tube A, which were also not seen to the same extent in the RP-HPLC chromatogram of tube M matrix. These peaks were not further identified in the frame of this study. Thus, the origin of these peaks and their identity cannot finally be confirmed. However, it is assumed that these peaks indicate additional unbound chemical compounds other than the BPA, BADGE and the BADGE derivatives studied. This assumption is supported by the extraction profile of tube A which was obtained by extracting the empty tubes with acetonitrile [4]. In this chromatogram a large number of additional peaks beside BPA, BADGE and its derivatives could be detected. The results with Macrogol/cetyl alcohol matrix stored in tube A suggest that these multiple extractable compounds are also capable to migrate from the inner lacquer of tube A into the ointment. Similar results were also obtained with *Macrogol Ointment* stored in tube A (data not shown).

Thus, storage of the matrix in tube A does not only result in a higher contamination of the ointment by BPA, BADGE and BADGE derivatives but overall in a significant higher amount of chemical compounds which are not present in the ointment when stored in tube M.

In view of the reactivity of the epoxy structure of BADGE, however, at least some of these peaks might also indicate reaction products of BADGE and formulation components. This hypothesis is supported by the fact that peak detection was conducted by fluorescence measurement (excitation at UV 275 nm and emission at 305 nm), which can be considered selective for molecules with structures similar to BADGE or BPA. Furthermore, Petersen et al.

Table 4
Leachates in *Macrogol Ointment* stored in aluminium tube type M at 40 °C for 8 weeks and under simulated in-use conditions compared with the amounts extracted by means of acetonitrile at 40 °C for 10 days (**data calculated in µg/L from Table 2).

Analytes	BADGE 2 H_2O	BPA	BADGE H_2O	BADGE·H_2O·HCl	BADGE	BADGE·HCl
	Macrogol Ointment by migration (µg/kg matrix)					
Day 56	<LOQ	54.2 ± 19.6	<LOQ	<LOD	<LOQ*	<LOD
In-use	<LOQ	127 ± 8	<LOQ	<LOD	<LOQ	<LOD
	*Acetonitrile extraction (µg/L)***					
	50.5 ± 8.4	307 ± 20	126 ± 40	<LOD	148 ± 63	<LOD

Mean and SD of samples tested (migration study, $n = 3$; extraction study, $n = 5$; *$n = 2$)

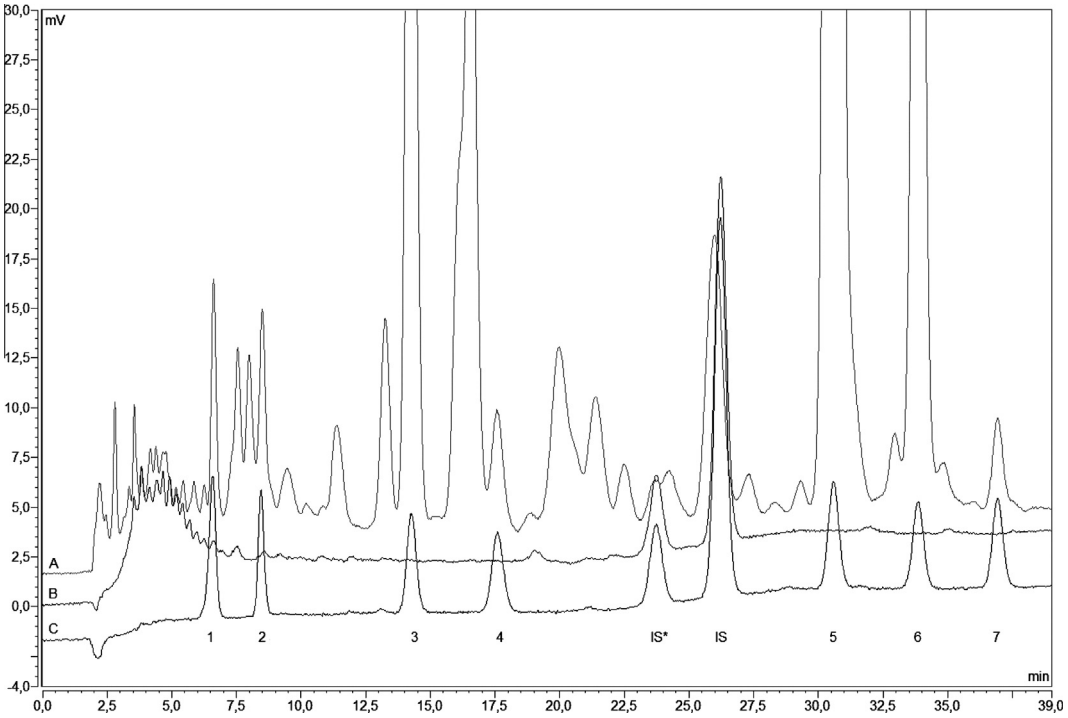


Fig. 3. RP-HPLC chromatogram of Macrogol/cetyl alcohol matrix stored in tube A (line A) and in tube M (line B) and BADGE standard solution (line C) for reference. 1, BADGE·2H_2O; 2, BPA; 3, BADGE·H_2O; 4, BADGE·H_2O·HCl; 5, BADGE; 6, BADGE·HCl; 7, BADGE·2HCl; IS, internal standard (BADPHE); IS*, impurity of BADPHE.

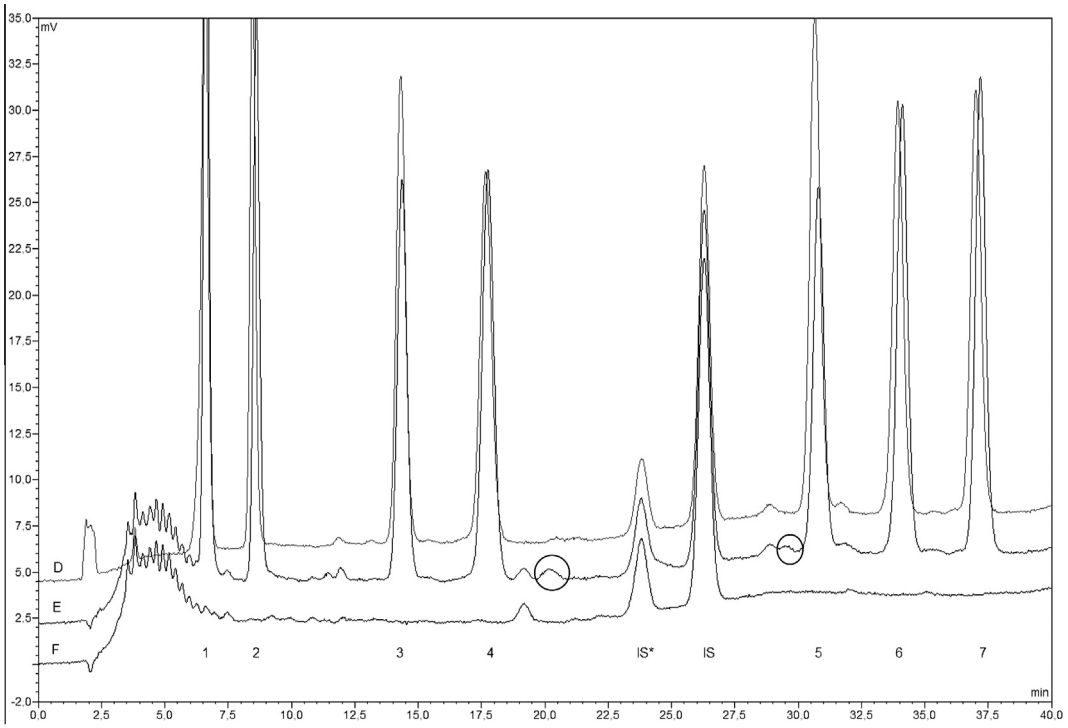


Fig. 4. RP-HPLC chromatogram of Macrogol/cetyl alcohol matrix stored in glass vials with (line E) and without (line F) addition of standard solution. The circles indicate the changes in the spiked matrix within 19 days of storage only. Line D shows the standard solution used for spiking. 1, BADGE·2H_2O; 2, BPA; 3, BADGE·H_2O; 4, BADGE·H_2O·HCl; 5, BADGE; 6, BADGE·HCl; 7, BADGE·2HCl; IS, internal standard (BADPHE); IS*, impurity of BADPHE.

[13] already reported on the reactivity of BADGE with food components after their migration from coated cans used in food packaging.

In order to verify that BADGE and its derivatives can react with components of the matrix, the Macrogol/cetyl alcohol matrix was spiked with the standard solution and was stored for 19 days in

glass containers (Fig. 4, line E) in comparison with the unspiked matrix (line F). In the chromatogram of the spiked matrix (line E), the peaks corresponding to BPA, BADGE and BADGE derivatives fully comply with the peaks in the chromatogram of the pure standard solution (Fig. 4, line D) at comparable concentrations. Moreover, additional peaks emerged in the spiked matrix after 19 days of storage (line E, indicated by circles). These additional peaks suggest that BADGE and reactive BADGE derivatives underwent reaction with components of the Macrogol/cetyl alcohol matrix. This assumption is also supported by the fact that after 19 days of storage the peak areas of BADGE (peak 3) and BADGE 1 H2O (peak 5) were reduced in the chromatogram of the spiked ointment. BADGE and BADGE 1 H2O are BADGE derivatives with at least one free epoxide group and thus capable to react easily with a second chemical entity. The peak areas of all other derivatives did not alter compared to the peaks of the standard solution (line D).

In consequence, storage of semi-solid dosage medicinal products in aluminium tube A with a high level of unbound BADGE and BPA in the inner lacquer cannot only result in a nearly exhaustive migration of the polymerisation residues from the coating into the medicinal product but may additionally lead to a contamination of the product by other unidentified extractable compounds as well as by reaction products between BADGE and/or reactive BADGE derivatives with formulation components. This contamination of the semi-solid formulation by leachables and potential reaction products impacts the purity of the formulation and thus has a negative effect on medicinal product quality.

Based on data from an in vitro skin permeation study and from the literature Søeborg et al. [14] concluded that the immediate human risk of BADGE and derivatives in topical dosage forms is low. The Estimated Systemic Exposure Dosage was found significantly below the established Total Daily Intake. However, for a comprehensive risk assessment on BADGE in topical medicinal product, not only systemic toxicity is to be taken into account, but also the allergenic potential of BADGE and BADGE derivatives. Oligomers of BADGE with a mean molecular weight $\leqslant$700 Da are the most frequent cause of contact allergy [15]. Recently, it was demonstrated that BADGE present in *Macrogol Ointment* in a quantity as determined after storage in tube A can induce allergic skin reactions when applied by epoxy-resin positive patients [16].

5. Conclusion

The magnitude of leaching predominantly depends on the composition of the semi-solid formulation. Components containing oxyethylene units turned out to have extractive properties, resulting in an almost quantitative release of unbound BADGE, BADGE derivatives and BPA from the inner coating of a tube into the medicinal product. Polyethylene glycol (PEG) based matrices, such as *Macrogol ointment*, are often used as a base for iodine or lidocaine containing medicinal products for cutaneous application.

Thus, in terms of medicinal product quality and safety, leaching of BADGE from the coating of the primary packaging into the medicinal product should be minimised as far as possible. As leaching is affected by formulation components contamination of a semi-solid medicinal product can only be avoided by using aluminium tubes with a low level of non-polymerised BADGE, BPA and BADGE derivatives. The European Standards EN 15384 and EN 15766 established for evaluating the porosity and polymerisation of the internal coating of tubes cannot replace appropriate extraction studies as described by Haverkamp et al. [4]. By means of these extraction studies aluminium tubes with a low level of extractables should be selected for medicinal product packaging in order to ensure the quality of semi-solid medicinal products up to their end of shelf life.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

[1] H.Q. Pham, M.J. Marks, Epoxy Resins. Ullmann's Encyclopedia of Industrial Chemistry. Electronic Release, Wiley-VCH, Weinheim, 2005.
[2] J. Simal-Gándara, S. Paz-Abuin, L. Ahrne, A critical review of the quality and safety of BADGE-based epoxy coatings for cans: implications for legislation on epoxy coatings for food contact, Crit. Rev. Food Sci. Nutr. 38 (8) (1998) 675–688.
[3] Commission Regulation (EC) No. 1895/2005 on the Restriction of Use of Certain Epoxy Derivatives in Materials and Articles Intended to Come Into Contact With Food, 2005, OJ L 302/28-32.
[4] J.B. Haverkamp, U. Lipke, T. Zapf, R. Galensa, C. Lipperheide, Contamination of semi-solid dosage forms by leachables from aluminium tubes, Eur. J. Pharm. Biopharm.: Official J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik eV 70 (3) (2008) 921–928.
[5] T. Søeborg, S.H. Hansen, B. Halling-Sørensen, Determination of bisphenol diglycidyl ethers in topical dosage forms, J. Pharm. Biomed. Anal. 40 (2) (2006) 322–330.
[6] R. Voigt, Pharmazeutische Technologie, Deutscher Apotheker Verlag, Stuttgart, Germany, 2010.
[7] DIN EN 15766:2009, Packaging – Flexible Aluminium Tubes – Test Methods to Determine the Polymerization of the Internal Coating with Acetone, Beuth Verlag GmbH, Berlin, Germany.
[8] DIN EN 15384:2007, Packaging – Flexible Aluminium Tubes – Test Method to Determine the Porosity of the Internal Coating, Beuth Verlag GmbH, Berlin, Germany.
[9] Deutscher Arzneimittel-Codex (DAC; German Drug Codex), Govi-Verlag Pharmazeutischer Verlag GmbH, Eschborn, 2014.
[10] Guideline on Stability Testing of New Drug Substances and Products (ICH Q1A (R2)), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2003.
[11] DIN 32645:1994-05, Chemical Analysis; Decision Limit; Detection Limit and Determination Limit; Estimation in Case of Repeatability; Terms, Methods, Evaluation, Beuth Verlag GmbH, Berlin, Germany.
[12] Guideline on Validation of Analytical Procedures: Text and Methodology (ICH Q2 (R1)), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005.
[13] H. Petersen, A. Schaefer, C.A. Buckow, T.J. Simat, H. Steinhart, Determination of Bisphenol A diglycidyl ether (BADGE) and its derivatives in food: identification and quantification by internal standard, Eur. Food Res. Technol. 216 (2003) 355–364.
[14] T. Søeborg, L.H. Basse, B. Halling-Sørensen, Risk assessment of topically applied products, Toxicology 236 (1–2) (2007) 140–148.
[15] A. Thorgeirsson, S. Fregert, O. Ramnäs, Sensitisation capacity of epoxy resin oligomers in the guinea pig, Acta Derm.-Venereol. 58 (1978) 17–21.
[16] K. Breuer, C. Lipperheide, U. Lipke, T. Zapf, H. Dickel, R. Treudler, et al., Contact allergy induced by Bisphenol A diglycidyl ether leachables from aluminium tubes for pharmaceutical use, Eur. J. Allergy Clin. Immunol. 70 (2) (2015) 220–226.
[17] Commission Regulation (EU) No. 10/2011 on Plastic Materials and Articles Intended to Come Into Contact With Food, 2011, OJ, L 12/1, Last Amended by Commission Regulation (EU) No. 2015/174, 2015, OJ L 30/2.